PROJECT NUMBER: 1730
PROJECT TITLE: Plant, Cell & Tissue Culture Research
PROJECT LEADER: I. L. Uydess
WRITTEN BY: M. Shulleeta
PERIOD COVERED: January, 1989

I. TOBACCO-IDENTICAL PRESERVATIVES:

A. **Objective**: To develop procedures and to establish microbiological screens for the evaluation of new, nature-identical preservatives as replacements for and/or as adjuncts to propylparaben.

B. **Status**:

1. Phase I Screens

Several shake-flask experiments were conducted to compare the antimicrobial effect of propylparaben (PP) in two different carriers; triethylene glycol (TEG) and propylene glycol (PG). Stock solutions of propylparaben were prepared in 10% TEG and PG and employed at doses of 75 and 150 µg/ml against the target organism, BC-13 (B. coagulans). The results of each experiment indicated that there were no significant differences between the activity of propylparaben in TEG vs PG at the lowest concentration tested (75 µg/ml) and only a marginal increase in effectiveness (~10-15%) using TEG as the carrier for propylparaben at 150 µg/ml. In each case, however, (150 µg/ml PP-TEG vs PP-PG) only about a half-maximal inhibition of growth was obtained.

A number of additional C-9 to C-12 compounds (lacking nitrogen) have been identified by R. Southwick and R. Izac as logical candidates for screening in the alternate preservatives program. The selection was based upon the observations made thus far regarding the efficacy of the C-9 thru C-12 fatty acids. The compounds selected include:

a. norsolanadione
b. 3-phenylproprionic acid
c. allyl caproate
d. carvacrol
e. methyl-2-nonenoate
f. ethyl phenylacetate
g. solanesol
h. decanol
i. β-cyclocitrylidene acetic acid (and the sodium salt)

2. Phase III Screens

Two dose-response experiments were performed in 5-liter fermentors in order to determine the minimum concentration of decanoic acid necessary to inhibit the gross chemical changes associated with the laboratory-induced spoilage of SEL. A change in pH was used as a general indicator of the chemical modification of the

SEL. Individual samples of SEL were also submitted to the Analytical Research Division for specific determinations of nitrate, nitrite, total reducing sugars and selected organic acids. Doses of 0, 150, 300 and 400 µg/ml decanoic acid (in PG) were evaluated. The results demonstrated that 150 µg/ml decanoic acid inhibited major chemical changes for up to 12 hours. However, 300 µg/ml of decanoic acid was required to inhibit changes in pH for 24 to 48 hours, or longer (the analytical data were not available at the time of this writing).

C. **Conclusions**:

1. Relatively low doses of decanoic acid (~150 µg/ml) can be employed to inhibit the progression of major chemical changes in SEL for 8 - 12 hours, but higher doses (in the order of 300 µg/ml) are required to inhibit the progression of spoilage for 24 hours or longer based upon a change in pH.

D. **Plans**: **February, 1989**

1. Initiate Phase I screening of new preservative candidates.

2. Continue to optimize the Phase III fermentor screen.

3. Conduct additional Phase III screens of decanoic acid, propyl-paraben and sorbate as individual compounds and in mixtures.